Amendments to the Specification:

Please replace the paragraph at page 1, beginning at line 3 with the following amended paragraph:

This application is a continuation of Serial No. 09/069,827, now pending, which is a continuation-in-part of Serial No. 09/050,359, now pending, which is a continuation-in-part of PCT/US97/19638, filed October 31, 1997, now ~~pending~~ abandoned, which is a continuation-in-part of Serial No. 08/740,671, filed October 31, 1996, now ~~pending~~ abandoned, which applications are hereby incorporated by reference in their entirety.

Please replace the paragraph at page 2, beginning at line 20 with the following amended paragraph:

Peptides have been found to bind proteins at the same sites as those by which the proteins interact with other proteins, macromolecules and biologically significant substances e.g. nucleic acids, lipids and enzyme substrates. The first examples of this property were in the publications of several groups who showed that there is a single peptide binding site on the biotin binding protein streptavidin. This is the same site responsible for biotin binding and these peptides compete with biotin for binding to this site (Biochemistry 34: 15430-15435 (1995) Screening of cyclic peptide phage libraries identifies ligands that bind streptavidin with high affinities, L. B. Giebel, R. T. Cass, D. L. Milligan, D. C. Young, R. Arze & C. R. Johnson; Gene 128: 59-65 (1993) An M13 phage library displaying random 38-amino-acid peptides as a source of novel sequences with affinity to selected targets, B. K. Kay, N. B. Adey, Y. S. He, J. P. Manfredi, A. H. Mataragnon & D. M. Fowlkes; Nature 354: 82-4 (1991) A new type of synthetic peptide library for identifying ligand-binding activity Sepetov, et al. ~~K. S. Lam, S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M. Kazmierski & R. J. Knapp~~; Proc Natl Acad Sci U S A 92: 5426-5430 (1995) Library of libraries: approach to synthetic combinatorial library design and screening of "pharmacophore" motifs, I. Saggio and R. Laufer ~~N. F. Sepetov,~~

~~V. Krchnak, M. Stankova, S. Wade, K. S. Lam & M. Lebl~~; Biochem J 293 (Pt 3): 613-6 (1993) Biotin binders selected from a random peptide library expressed on phage, I. Saggio & R. Laufer). Many other examples exist, for instance Smith demonstrated that peptides displayed on phage which bound to ribonuclease S had a specific consensus motif and that these PLs were antagonistic to ribonuclease activity, implying that the peptides and the RNA were bound by the same ligand binding site (Gene 128: 37-42 (1993) A ribonuclease S-peptide antagonist discovered with a bacteriophage display library, G. P. Smith, D. A. Schultz & J. E. Ladbury). Another example is from the binding of peptide ligands to cell surface integrins (Biochemistry 34: 3948-3955 (1995) Peptide ligands for integrin alpha v beta 3 selected from random phage display libraries, J. M. Healy, O. Murayama, T. Maeda, K. Yoshino, K. Sekiguchi & M. Kikuchi; J Cell Biol 124: 373-80 (1994) Isolation of a highly specific ligand for the alpha 5 beta 1 integrin from a phage display library, E. Koivunen, B. Wang & E. Ruoslahti). Peptides obtained in this way clearly mimic natural protein:protein interactions as in the case for the proteins MDM2 and p53 (Bottger et al. Identification of novel mdm2 binding peptides by phage display, Oncogene, 13:2141-7 (1996)). However, it has not hitherto been appreciated that this phenomenon is sufficiently common so that it might be exploited in identifying inhibitors of the interaction of a protein with an unknowing binding partner. Nor have others explained just how to take advantage of this phenomenon for that purpose.

Please replace the paragraph at page 5, beginning at line 30 with the following amended paragraph:

Libraries of proteins (Ladner, USP 5,223,409 (07/664,989, filed March 1, 1991) ~~4,664,989~~), peptoids (Simon *et al.*, Proc Natl Acad Sci U S A, 89:9367-71(1992)), nucleic acids (Ellington and ~~JW~~ Szostak, Nature, 246:818(1990)), carbohydrates, and small organic molecules (Eichler *et al.*, Med Res Rev, 15:481-96(1995)) have also been prepared or suggested for drug screening purposes.

Please replace the paragraph at page 7, beginning at line 32 with the following amended paragraph:

DGI Technologies, WO96/04557 corresponding to Blume, USP 6,010,861, "Target Specific Screens and Their Use for Discovering Small Organic Molecular Pharmacophores", suggests first screening a library composed of mutated variable domains of antibodies (V-H, V-L, or single chain antibodies, which are V-H and V-L domains joined by a peptide linker) for domains which bind the target. Preferably, the parental variable domains have a solved 3D structure (p. 44).

Please replace the paragraph at page 10, beginning at line 1 with the following amended paragraph:

Preferably, the peptides of the first library are of 5-50 amino acids. Peptides of at least five amino acids length ~~lengthh~~ are sufficiently large to bind to an active site with a reasonably high activity. Peptides larger than about 50 amino acids are generally too large to fit into an active site, and also are more cumbersome to synthesize.

Please replace the paragraph at page 10, beginning at line 20 with the following amended paragraph:

For the foregoing reasons, when the first combinatorial library is composed of peptides, the peptides do not comprise an antibody-like domain. Thus, the peptides cannot be antibodies, single chain antibodies, or isolated variable heavy or light domains thereof. This exclusion applies both to naturally occurring antibodies, and to mutant antibodies which retain the normal structure of an antibody variable domain. The term "antibody-like domain" refers to a peptide having the normal structure of ~~of~~ an antibody variable domain, as defined at col. 15, lines 59-68 of Ladner, USP 5,403,484.

Please replace the paragraph at page 15, beginning at line 35 with the following amended paragraph:

For each of 16 targets, the sequences of the binding

peptides ~~petpides~~ identified by screening phage display libraries were analyzed. First, for peptides binding a given target, a consensus sequence was determined, and from this, a core binding region. The amino acids in the core region of all the peptides binding that target were tallied, and the tallies were divided by the number of peptides in question, to obtain a subtotal for each target. The target subtotals were then added and divided by the number of targets. The final totals were converted into percentages. If all of the residues were represented equally their values would be 5%.

Please replace the paragraph at page 31, beginning at line 15 with the following amended paragraph:

1 GAX NNK NNK NNK NNK TGG NNK NNK NNK NNK NNK (SEQ ID NO:1)
2 NNK GAX NNK NNK NNK TGG NNK NNK NNK NNK NNK (SEQ ID NO:2)
3 NNK NNK GAX NNK NNK TGG NNK NNK NNK NNK NNK (SEQ ID NO:3)
4 NNK NNK NNK GAX NNK TGG NNK NNK NNK NNK NNK (SEQ ID NO:4)
5 NNK NNK NNK NNK GAX TGG NNK NNK NNK NNK NNK (SEQ ID NO:5)
6 NNK NNK NNK NNK NNK TGG GAX NNK NNK NNK NNK (SEQ ID NO:6)
7 NNK NNK NNK NNK NNK TGG NNK GAX NNK NNK NNK (SEQ ID NO:7)
8 NNK NNK NNK NNK NNK TGG NNK NNK GAX NNK NNK (SEQ ID NO:8)
9 NNK NNK NNK NNK NNK TGG NNK NNK NNK GAX NNK (SEQ ID NO:9)
10 NNK NNK NNK NNK NNK TGG NNK NNK NNK NNK GAX (SEQ ID NO:10).

Please replace the paragraph at page 37, beginning at line 26 with the following amended paragraph:

If the peptide library is in a solution phase, the TP may be immobilized, and the library screened by the method of Cantley, infra. The target may be immobilized on chromatographic media either directly, e.g., using AFFIGEL matrix ~~Affigel~~ (BioRad), or indirectly. In indirect immobilization, the TP is noncovalently conjugated to the support by means of an affinity reagent. For example, target protein tagged with six histidines may be immobilized on QIAGEN ~~Qiagen~~ nickel binding resin, or a GST (glutathione S-transferase) tagged target immobilized on

glutathione SEPHAROSE ~~Sepharose~~ chromategraphy matrix (Pharmacia), or a maltose binding protein/target protein fusion immobilized on maltose (New England Biolabs) or dextran media. Subsequently, one uses the immobilized target to separate out peptides with desired activity by the method of Cantley et. al. (Trends Biochem. Sci. 20: 470-475 (1995) [96108162] Recognition and specificity in protein tyrosine kinase-mediated signalling.S. Zhou & L. C. Cantley and Methods Enzymol 254: 523-535 (1995) [96052729] SH2 domain specificity determination using oriented phosphopeptide library. S. Zhou & L. C. Cantley and Cell 72: 767-78 (1993) [93201599] SH2 domains recognize specific phosphopeptide sequences. S. Zhou, S. E. Shoelson, M. Chaudhuri, G. Gish, T. Pawson, W. G. Haser, F. King, T. Roberts, S. Ratnofsky, R. J. Lechleider &...). In this method a mixture of peptides are eluted from the TP and the whole mixture sequenced by automated techniques. Useful information is most readily achieved by using biased peptide library pools wherein one amino acid resident is "fixed", for example, x-x-x-x-Y-x-x-x-x-x (SEQ ID NO:11). If a tyrosine (Y) is in fact contained with a binding motif for a domain contained within the TP, then other residues within the motif will be in register with the tyrosine. Thus, if cognate binding peptides must have a leucine following the tyrosine, then the sequencing of the pool will yield a sequence of x-x-x-x-Y-L-x-x-x-x (SEQ ID NO:12). Without *a priori* knowledge of the natural binding partner, this makes it preferable to use up to 19 (all amino acids except for cysteine) library pools. This is not really so overwhelming as the costs of the random peptide pools is not prohibitive. It is preferable to avoid cysteine as a component as peptides with an odd number of cysteines do not bind well.

Please replace the paragraph at page 59, beginning at line 27 with the following amended paragraph:

To circumvent this problem we have taken a novel approach. The consensus sequences for ligands have at least one amino acid

residue which is highly conserved. Purpose built libraries (i.e. a X-X-X-P-P-X-X-P-X-X (SEQ ID NO:13) library (Yu H, Chen JK, Feng S, Dalgarno DC, Brauer AW and Schreiber SL (1994) Cell 76:933-945)) based on consensus ligand sequences have been successfully used to isolate large numbers of phage displaying binding peptides using proteins in the same family. We theorized that libraries which have a single fixed residue flanked by regions coding for a random peptide should work in a similar fashion for targets for which no information on peptide ligands is available. If the fixed residue is important in the formation of a ligand, the number of phage which will display a peptide with binding characteristics will be enriched. Conversely, if the fixed residue is deleterious for the formation of a binding peptide, the number of phage in this library which display binding characteristics will be reduced.

Please replace the paragraph at page 60, beginning at line 18 with the following amended paragraph:

3) a $X_6PXPPXPX_2$ (SEQ ID NO:14) motif which conforms to the consensus for ligands that bind SH3 domain containing proteins (Class I SH3 purpose built library).

Please replace the paragraph at page 72, beginning at line 25 with the following amended paragraph:

Like most HCMV mRNAs, the mRNA encoding UL44 is not spliced. Therefore, it is possible to isolate the complete UL44 coding sequence as a functional unit from HCMV genomic DNA prepared from purified virions. We have used the following oligonucleotide primers and the PCR to amplify the coding region of UL44 from CMV DNA: 5'-CTGTGCGGATCCATGGATCGCAAGACG-3' (SEQ ID NO:15) and 5'-CTGTGCGAATTCCTAGCCGCACTTTTG-3' (SEQ ID NO:16). The resulting 1.3 kb product was purified using a WIZARD PCR PREPS ~~Wizard PCR Preps~~ clean up resin, blunted with T4 DNA Polymerase (NEB), cut with BamHI repurified on an agarose gel and cloned into the vector pGex2T digested with BamHI and SmaI. Individual clones were

tested for the correct insert by restriction enzyme digests and two clones were sequenced in entirety to insure the correct protein was encoded by the clone. E. coli DH5aF' carrying the plasmid were induced with IPTG to produce the fusion protein and the product was purified by affinity chromatography using glutathione SEPHAROSE matrix ~~sepharose~~ as recommended by the manufacturer (Pharmacia). The resulting protein was used as a fusion protein or was cleaved from the glutathione ~~sepharose~~ SEPHAROSE chromatography matrix using the protease thrombin (which cleaves between the GST portion of the fusion and the UL44 protein) by treating 1 mg of fusion protein on beads with 50 Units of Thrombin (Pharmacia) for 2 hours at room temperature. The resulting UL44 protein was analyzed by SDS gel electrophoresis and contained two major cleavage products, one of full length and the other roughly 5 kdaltons smaller.

Please replace the paragraph at page 73, beginning at line 14 with the following amended paragraph:

Phage libraries were made using published protocols (Construction of Random Peptide Libraries in Bacteriophage M13 in Phage Display of Peptides and Proteins: A Laboratory Manual. Edited by B. Kay, J. Winter and J. McCafferty. Academic Press 1996.). Briefly, oligonucleotides which encoded the random peptide with one residue fixed were converted to double stranded DNA by extending a complementary primer using ~~Sequenase~~ SEQUENASE modified T7 DNA polymerase (USB). The resulting fragments were digested with XhoI and XbaI, gel purified and ligated into previously digested mBAX vector. The ligation was introduced into bacteria by ten successive electroporations and the transformed bacteria were amplified overnight. The supernatant containing phage was harvested and the phage precipitated using PEG/NaCl, resuspended in 1X PBS containing 10% glycerol and frozen at -80° C. Ten of the oligonucleotides encoded peptides with the following structure: X_5UX_5 where X is any amino acid and U is a fixed residue. The following residues were fixed, one in

each library: D(GAT), F(TTC), H(CAC), K(AAA), L(CTG), M(ATG), N(AAT), P(CCG), R(CGT), and W(TGG). For convenience, the X_5DX_5 library is referred to as a "D" library, X_5FX_5 as an "F" library, etc. The oligonucleotide sequence for each was 5'-GACTGTGCCTCGAGK(NNK)$_5$xxx(NNK)$_5$TCTAGACGTGTCAGT-3' (SEQ ID NO:17) where xxx is the codon shown above for each residue fixed. In addition, a library with 10 random residues followed by a fixed C (TGC) was constructed with the same flanking sequences. This is referred to as the "$X_{10}C$" library. The oligonucleotide with the sequence of 5'-ACTGACACGTCTAGA-3' (SEQ ID NO:18) was used as the primer to convert the ssDNA to double stranded.

Please replace the paragraph at page 74, beginning at line 9 with the following amended paragraph:

UL44 or GSTUL44 was immobilized on microtiter plates (Costar) by incubating 1 μg of protein in 200 μl of 0.1 M $NaHCO_3$, pH 8.5 overnight at 4° C. The remaining protein binding sites on the plate were blocked by adding 150 μl of 1% BSA in 0.1 M $NaHCO_3$ and incubating the plate at room temperature for 1 hour. The plate was then washed 5 times with 300 μl of TBST (100 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.1 % ~~tween~~ TWEEN-20 detergent). Phage libraries were then added to the wells in 200 μl of TBST and allowed to incubate at room temperature for 5 hours. The wells were washed 5X with TBST and the phage were eluted by incubating with 200 μl of 50 mM Glycine, pH 2.0 for 10 minutes. The eluant was removed to a tube at the pH neutralized with 200 μl of 200 mM NaHPO4 buffer, pH 7.0. The ~~the~~ phage were then amplified by adding the eluted phage to 5 ml of 2XYT broth containing 1:100 dilution of an overnight culture of E. coli DH5αF'. The cultures were grown with agitation overnight at 37° C. The next morning the bacteria were removed by centrifugation at 3000 xg for 10 minutes in a SS-34 rotor. 100 μl of the supernatant containing the amplified phage were then used in the next round of affinity purification.

Please replace the paragraph at page 75, beginning at line 28 with the following amended paragraph:

Isolated from panning against GSTUL44 and thrombin cleaved UL44 on ~~Immulon4~~[R] IMMULON4[R] microtitration test plates as well as thrombin cleaved GSTUL44 on ~~Covalink~~[R] COVALINK[4] microbiological and immunological analysis plates

$X_{10}C$	E	H	V	C	S	W	G	W	G	R	**C** (SEQ ID NO:19)
others not from $X_{10}C$:											
17	P	T	S	D	L	**W**	R	N	L	G	G (SEQ ID NO:20)
18	W	G	E	T	M	**W**	D	N	R	K	V (SEQ ID NO:21)
11 many clones	A	G	L	T	P	**W**	S	L	L	V	D (SEQ ID NO:22)
8H	D	T	G	T	W	**W**	H	S	Y	V	L (SEQ ID NO:23)
2A	R	A	P	L	A	**D**	R	L	L	E	G (SEQ ID NO:24)
2E	K	L	W	S	A	**D**	M	S	S	I	V (SEQ ID NO:25)
2H	F	I	V	G	N	**D**	Y	R	L	G	K (SEQ ID NO:26)
8B	E	G	Y	P	S	**W**	V	Y	M	G	M (SEQ ID NO:27)
1E	A	R	D	F	E	**D**	V	Q	Q	C	C (SEQ ID NO:28)

Please replace the paragraph at page 77, beginning at line 5 with the following amended paragraph (here, inserts are bolded as well as underlined as underlining appears in the original text):

IMMULON 4 ~~Immulon 4~~ (cat.# 011-010-3855) 96-well **microtitration test** plates were purchased from Dynatech. Bovine serum, albumin (BSA) (A2153), Streptavidin alkaline phosphatase (SA-AP) (S2890), ~~Tween-20~~ **TWEEN-20 detergent** (P1379), and *p*-nitrophenyl phosphate tablets (*p*NPP) (N-1891, N-2770) were purchased from Sigma. Phosphate buffered saline (PBS) (21600-010) was obtained from Gibco-BRL. Ultrapure glycerol (#16374) was purchased from USB. Biotinylated surrogate ligands were prepared as a 1 mM stock solution in the appropriate solvent (H_2O or 10% acetonitrile). SA-AP was prepared as a 1 mg/ml stock solution in PBS containing 10% glycerol and stored in aliquots at -80 °C. Peptides corresponding to peptides displayed on the surface of binding phage (H-Ser-Gly-Ser-Gly-Glu-His-Val-Cys-Ser-

Trp-Gly-Trp-Gly-Arg-Cys-OH **(SEQ ID NO:29)** and Biotin- H-Ser-Gly-Ser-Gly-Glu-His-Val-Cys-Ser-Trp-Gly-Trp-Gly-Arg-Cys-OH **(SEQ ID NO:30)**) (the underlined residues were from the $X_{10}C$ peptide listed above; the Ser-Gly-Ser-Gly unit is a linker) were synthesized by AnaSpec, Inc.

Please replace the paragraph at page 77, beginning at line 22 with the following amended paragraph:

Target protein was immobilized in microtiter wells by incubating 0.5-2.0 µg per well in 100 µl of 0.1 M $NaHCO_3$ overnight at 4 °C. (Studies have indicated that maximal protein binding can be attained with 0.5 µg of target protein per well.) The target protein was removed and the wells were blocked with 200 µl of 1% BSA prepared in 0.1 M $NaHCO_3$ for 1 hour at room temperature. During the 1 hour blocking period, the SA-AP:surrogate ligand conjugate was prepared by mixing 2 µg SA-AP and 50 pmol biotinylated surrogate ligand for each well of target protein. (This corresponds to a 1:1 ratio of biotinylated peptide to biotin binding sites.) The mixture was incubated at room temperature for 15-20 minutes and then diluted with Tris-buffered saline-~~Tween-20~~ TWEEN-20 detergent (TBST) 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% ~~Tween-20~~ TWEEN-20 detergent such that there was 100 µl of conjugate per well. For lower concentrations of surrogate ligand, an unrelated biotinylated peptide was titrated in to keep the total amount of biotinylated peptide at 50 pmol/well and total SA-AP at 2 µg/well.

Please replace the paragraph at page 80, beginning at line 17 with the following amended paragraph:

As discussed above, peptides generally do not cross the plasma membrane of cells. Although technology is available to deliver peptides into cells with high efficiency using liposomes, electroporation, micro-injection, etc., we plan to use an assay that does not depend on the intracellular delivery of peptides as our initial method to evaluate the physiological effect of the

peptides that interact with the processivity factor. This *in vitro* assay was originally developed in the HSV-1 system (Hernandez and Lehman, 1990), and we will adapt and validate the assay for the HCMV replication system. The *in vitro* HSV-1 system utilizes three virus-coded proteins: the DNA polymerase (HSV UL30), the processivity factor (HSV UL42), and the single-stranded DNA-binding protein (HSV UL29). The template used for the *in vitro* replication system is single-stranded M13mp18 DNA to which an oligonucleotide primer (5'-GTTTTCCCAGTCACGAC-3' (SEQ ID NO:31)) is annealed. The oligonucleotide is generally used for DNA sequencing and is commercially available (New England BioLabs). Thus, the replication assay is an origin-independent primer extension reaction. The HSV-1 polymerase and single-stranded DNA-binding protein extend the primer annealed to M13 DNA, producing chains of variable length and only a small quantity of completely double-stranded DNA where the primer has been extended the full length of the M13 circular DNA. In contrast, with addition of the processivity factor, most of the product is full length, double-stranded M13 DNA. The production of variable length versus full length DNA products is monitored by the electrophoretic separation of reaction products in agarose gels (Hernandez and Lehman, 1990).

Please replace the paragraph at page 81, beginning at line 8 with the following amended paragraph:

Ertl and Powell (1992) showed that the HCMV polymerase and processivity factor, purified as recombinant proteins from baculovirus-infected Sf9 cells, cooperate in a primer extension reaction, and they further demonstrated that the processivity factor stimulated the activity of the polymerase in the assay. Similarly, Weiland *et al.*, (1994) have shown that recombinant HCMV UL44 processivity factor produced in *E. coli* can enhance the activity of HCMV DNA polymerase in a primer extension assay. We will implement the origin-independent primer extension assay in the HCMV system as follows. The coding sequence for the HCMV

polymerase (UL54) and single-stranded DNA-binding protein (UL57) will be amplified from HCMV genomic DNA as described above for the processivity factor (UL44). The UL54 and UL57 coding regions will be validated by automated DNA sequence analysis, and cloned into pBlueBacHis2 (Invitrogen). The plasmids will then be used to construct baculovirus recombinants for expression of the replication proteins in Sf9 insect cells. Expressed proteins carry two tags at their 5' ends, one the Xpress leader peptide (Asp Leu Tyr Asp Asp Asp Asp Lys (SEQ ID NO:178)) is easily detected with a monoclonal antibody in ELISA assays and the other includes a six histidine binding site that has a high affinity for divalent cations. Nickel-chelating resins will allow us to purify the recombinant proteins in one step. The HCMV equivalent of the HSV-1 replication assay will then be optimized using purified proteins.

Please replace the paragraph at page 81, beginning at line 33 with the following amended paragraph:

When we have successfully implemented the *in vitro* origin-independent primer extension assay, we will titrate peptides that we have shown to interact with the processivity factor into the reaction to test for their ability to perturb the interaction. It is possible that most if not all peptide-processivity factor interactions will be of considerably lower affinity that the polymerase-processivity factor or DNA-processivity factor interaction, but we can use a vast excess of the peptide to drive the peptide interaction to search for effects. We can also vary the order of addition of reactants to give the peptide an opportunity to interact with the processivity factor before the addition of other factors. We anticipate that some of the peptides will interfere with the essential interactions of the processivity factor; and, as a result, will inhibit processivity in the primer extension reaction. The specificity of inhibitory effects will be assayed by testing the ability of active peptides to inhibit the activity of mammalian DNA polymerase alpha

(partially purified from HeLa cells by sequential chromatography on Q-~~Sepharose~~ SEPHAROSE chromatography matrix and double-stranded DNA cellulose, Owsianka et al., 1993) or the *E. coli* Klenow polymerase (commercially available) on the M13-primer complex. There is precedent for this type of assay in the HSV-1 system. Owsianka *et al.* (1993) assayed a series of 15-mer peptides corresponding to segments of the HSV-1 processivity factor (the opposite 'sense' to our peptides that will bind to the processivity factor), and identified one peptide that inhibited a primer extension reaction and exhibited some specificity for the viral as compared to the cellular DNA polymerase. The peptides that inhibit the HCMV replication reaction would then be used in high throughput peptide displacement screens for identification of small molecules from combinatorial libraries with the potential to interfere with processivity in phase II of this proposal.

Please replace the paragraph at page 92, beginning at line 33 with the following amended paragraph:

Frequency									Sequence			
8	G	K	G	W	K	C	F	G	A	L	C	(SEQ ID NO:32)
2	S	T	T	F	Q	C	V	G	L	L	C	(SEQ ID NO:33)
1	A	N	G	W	E	C	I	G	Q	F	C	(SEQ ID NO:34)
1	K	P	V	W	K	C	T	G	L	F	C	(SEQ ID NO:35)
1	S	A	Q	W	Q	C	V	G	E	F	C	(SEQ ID NO:36)
1Consensus					W	Phi	C	Pho	G	x	F/L	C

Phi=hydrophilic
Pho=hydrophobic

2	L	P	M	A	R	W	T	C	V	N	C	(SEQ ID NO:37)
1	A	V	D	R	G	W	T	C	V	N	C	(SEQ ID NO:38)
1	Q	I	T	P	Q	W	T	C	I	N	C	(SEQ ID NO:39)
1Consensus							W	T	C	V/I	N	C
1	G	V	C	Q	S	S	D	H	R	E	C	(SEQ ID NO:40)
1	G	W	Q	E	R	F	Q	Q	E	D	R	(SEQ ID NO:41)
1	E	V	P	T	T	K	V	L	W	P	S	(SEQ ID NO:42)

Please replace the paragraph at page 96, beginning at line 7 with the following amended paragraph:

	PFQDYWEELLN	(SEQ ID NO:43)
	PFHSWWQDLTD	(SEQ ID NO:44)
	NFWDEWQTFMD	(SEQ ID NO:45)
11 clones	SFTDYWRDLEQ	(SEQ ID NO:46)
Consensus	xFxDyWqdLxx	(SEQ ID NO:47)

MEEPQSDPSVEPPLSQETFSDLWKLLPENNVL human p53 (SEQ ID NO:48)
MTAMEESQSDISLELPLSQETFSGLWKLLPPEDIL mouse p53 (SEQ ID NO:49)
surface contacts: * ** **

Other peptides which do not fit the consensus:

GAPWNWEKKEL (SEQ ID NO:50)
ADPRLPVEREL (SEQ ID NO:51)
MDGSGGERNSMW (SEQ ID NO:52)
PMRTEWAVGSES (SEQ ID NO:53)

Please replace the paragraph at page 98, beginning at line 38 with the following amended paragraph:

frequency	Screen	Library	Sequence (SR linker-random peptide-SR linker)	
22+9+2	1,2,3	$X_{10}C$	S R V C A I W P D L D G C S R	(SEQ ID NO:54)
6	1	$X_{10}C$	S R W C S L R P Q D E G C S R	(SEQ ID NO:55)
2	1	$X_{10}C$	S R W C E L W S Q D I G C S R	(SEQ ID NO:56)
11+6	2,3	P	S R W C E L W P E G S G C S R	(SEQ ID NO:57)
1	3	$X_{10}C$	S R L C E V W P Q T A G C S R	(SEQ ID NO:58)
1	3	P	S R W C D I W P D T G S C S R	(SEQ ID NO:59)
1	3	P	S R L C D I M P Q T V G C S R	(SEQ ID NO:60)
1	3	P	S R W C E V W P D K R W C S R	(SEQ ID NO:61)
CONSENSUS			S R W $C_{acidPho}$ W P $_{Phi\ x\ x}$ G C S R	(SEQ ID NO:62)

$X_{10}C$ TCG AGG GTG TGT GCT ATT TGG CCG GAT CTG GAT GGT TGC TCT AGA (SEQ ID NO:63)
S R V C A I W P D L D G C S R (SEQ ID NO:64)

P TCG AGG TGG TGT GAG TTG TGG CCG GAG GGT TCT GGT TGT TCT AGA (SEQ ID NO:65)
S R W C E L W P E G S G C S R (SEQ ID NO:66)

Please replace the paragraph at page 103, beginning at line 17 with the following amended paragraph:

# of Clones	Library	Sequence	
1	CWL	L **Y S W P** D E Q Y E R P(TyrRS1)	(SEQ ID NO:67)

1	W	F G F **Y** G **W** **P** D D Q Y (SEQ ID NO:68)
1	PHD12	M **Y** T **W** **P** G S P Y L Q M (SEQ ID NO:69)
2	PHD12	M **Y** S **W** **P** G E H Y T V H (SEQ ID NO:70)
2	CWL	M **Y** A **W** **P** D S S E L E K (SEQ ID NO:71)
1	PHD7	M **Y** S **W** **P** G V (SEQ ID NO:72)
2	PHD7	Y **Y** G **W** **P** S E (SEQ ID NO:73)
1	W	D R V **Y** G **W** **P** P F E E (SEQ ID NO:74)
1	W	A **Y** H **W** **P** W V E S E W (SEQ ID NO:75)
1	W	G **Y** S **W** **P** W P D D N A S R (SEQ ID NO:76)
1	W	I **Y** S **W** **P** W P S N E N (SEQ ID NO:77:)
1	PHD7	Q **Y** T **W** **P** W P (SEQ ID NO:78)
1	P	**Y** S **W** **P** W P D F N E T (SEQ ID NO:79)
1	W	A **Y** S **W** **P** W H D T V D (TyrRS2) (SEQ ID NO:80)
5	W	W D G F A **W** **P** M H Q T (SEQ ID NO:81)
3	F	**W** **P** W G G F E W P K L (SEQ ID NO:82)
1	D	R **Y** W **W** **P** D W G S R E (SEQ ID NO:83)
1	W	L W **W** **P** E W G V Y T G (SEQ ID NO:84)
3	D	Y F W **W** **P** D W G S S A (SEQ ID NO:85)
1	W	D R G W W **W** **P** S W G V S R (SEQ ID NO:86)
7	D	G **Y** W **W** **P** D W G S G Q (SEQ ID NO:87)
1	P	A E **Y** W **W** **P** D W G F F (TyrRS3) (SEQ ID NO:88)
1	W	R L Q **Y** W **W** **P** D W G P (SEQ ID NO:89)
4	N	M **Y** W **W** **P** N W G S Q E (TyrRS4) (SEQ ID NO:90)
1	P	W L D G L **P** L Y H E V (TyrRS5) (SEQ ID NO:91)
1	CWL	D T V R K D L L L E R E (TyrRS6) (SEQ ID NO:92)

Please replace the paragraph at page 104, beginning at line 1 with the following amended paragraph:

The peptide sequences are clustered in 4 distinct groups, the first two groups having multiple related members and the last two containing one sequence each. There are several similar positions in groups one and two: they all contain a central YXWP (SEQ ID NO:93) motif. It is tantalizing to speculate that the Y is mimicking free tyrosine and that the WP is mimicking ATP (with the P serving as the 5 membered sugar and the W as the base. A subset of group 1 has a W in the position immediately downstream of the conserved WP, however this is not universal. Group 2 on the other hand contains an extended conserved motif of YWWPDWG (SEQ ID NO:94) with a propensity for S in the next position.

Please replace the paragraph at page 111, beginning at line 22 with the following amended paragraph:

Affinity selections were carried out as in example 3 except that the protein was presented in several ways. At first the

protein was immobilized on ~~Immulon 4~~ IMMULON 4 microtitration test plates (Dynex) as in previous examples, however, repeated attempts failed to isolate phage which bound specifically to the target. It was found that the target exhibited greatly reduced activity when bound to ~~Immulon 4~~ IMMULON 4 plates, making it likely that it was denatured when bound to plastic. To circumvent this problem, two approaches were used, both of which utilized biotinylated protein. Protein (1 mg) was biotinylated with Sulfo NHS-LC-LC biotin (Pierce, cat. #21338) prepared fresh as a 10 mM stock solution in ddH_20. All proteins were in phosphate buffered saline. Biotinylation reagent was added to the protein solution in a 17-fold molar excess to protein and the reaction was carried out at room temperature for 30 minutes followed by ice for 30 minutes. Biotinylated proteins were separated from the excess biotinylation reagent on a SEPHADEX ~~Sephadex~~ G-50 chromatography matrix micro-spin column (~~Pharmaciea~~ Pharmacia Biotech, Cat# 27-5335-01) according to the manufacturers directions. Protein assays were conducted using BioRad Protein Assay reagent (cat#500-0006). All proteins were stored in 1X PBS+10% glycerol at -80° C.

Please replace the paragraph at page 112 beginning at line 32 with the following amended paragraph:

Displayed Peptide Sequence	Method	Library	Frequency
S S Q T D W R K I F Q S L S R (SEQ ID NO:95)	beads	K	3
S S S T D W L N V W R Q L S R (SEQ ID NO:96)	beads	N	2
S S A T D W G R V Y S I L S R (SEQ ID NO:97)	beads/sol	R	5
S S A S Y A P W P I Y F A S R (SEQ ID NO:98)	beads	W	2
S S G A F K P W P V Y S F S R (SEQ ID NO:99)	beads	W	1
S R Q V E V F K P W P V Y S R (SEQ ID NO:100)	beads/sol	K	3
S S S F K P W P I Y L G S S R (SEQ ID NO:101)	sol	P	1
S S E P F S V W P I Y K H S R (SEQ ID NO:102)	sol	W	1
S S S V P F A P W P V Y A S R (SEQ ID NO:103)	beads	P	1
S S T S L P F N R W P I Y S R (SEQ ID NO:104)	beads	N	2

Please replace the paragraph at page 113, beginning at line 34 with the following amended paragraph:

Displayed Peptide Sequence	Library	Frequency
S R L L E V S P G W W Q M S R (SEQ ID NO:105)	P	9

S S F R E L K P G W W S Y S R (SEQ ID NO:106)	P	1
S S W G D Y F N W R D G L S R (SEQ ID NO:107)	N	2

Please replace the paragraph at page 114 beginning at line 3 with the following amended paragraph:

Displayed Peptide Sequence	**Library**	**Frequency**
S R Q V E V F K P W P V Y S R (SEQ ID NO:108)	K	1
S S S F K P W P I Y L G S S R (SEQ ID NO:109)	P	1
S V S V G M K P S P R P (SEQ ID NO:110)	PHD12	2
S S N Y W W Q S P V L S R H S R (SEQ ID NO:111)	CWL	1
S S W Q G N V L L G N W I S R (SEQ ID NO:112)	L	3
S S L L N E S R L Q W S T S R (SEQ ID NO:113)	R	1

Please replace the paragraph at page 114 beginning at line 19 with the following amended paragraph:

Displayed Peptide Sequence	Library	Frequency
S R D W G F W D W G V D R S R (SEQ ID NO:114)	D	5
S R D W G F W R L P E S M A S R (SEQ ID NO:115)	CWL	3
S R E W H F W R D Y N P T S R (SEQ ID NO:116)	R	4
S S E R G S G D R G E K G S R (SEQ ID NO:117)	D	1

Please replace the paragraph at page 114 beginning at line 24 with the following amended paragraph:

These sequences are markedly different from those in example 4 above where the target was immobilized directly on ~~Immulon 4~~ IMMULON 4 microtitration test plates. It is likely that upon binding to plastic that the proteins conformation is altered and so may present a distorted binding site. Selection for phage which bind in solution may represent peptides which bind to a more native form of the protein and thus are a better indicator of the native conformation of the target.

Please replace the paragraph at page 123 beginning at line 12 with the following amended paragraph:

NGF-R LNGS**A**GDT**W**RHLAGE**LG**YQPEH**ID**SFTHE-----**A**CPVRA**LLA** cont'd below

Fas-R AGVMTLSQV**K**G**FVR**KN**G**VNEAK**ID**EIKND**N**VQDT**AE**QKVQ**LL**R cont'd below

TNF-R TDDP**A**TLY**WKEFVRRLG**LSDHE**ID**RLELQ**N**GRCLR**E**AQYSM**LA** cont'd below

NGF-R SWATQD--S**ATLDALLAALR**RIQRAD**LVE**SLCSEST**A**TSPV (SEQ ID NO:118)

Fas-R N**W**HQLHGKKEAY**DTLI**KD**L**KKAN**LCTLAEKI**QTIILKDITS (SEQ ID NO:119)

TNF-R T**W**RRRTRRE**ATLELLGRVLR**DMD**LL**GCL**E**DIEEALC**A**PPLP (SEQ ID NO:120)

Please replace the paragraph at page 124 beginning at line 33 with the following amended paragraph:

The libraries will be screened according to standard

techniques (Kay *et al.*, 1993; Adey and Kay, 1996. In brief, sever/al micrograms of GST-DD fusion protein will be immobilized in ELISA style microtiter plates. After non-specific protein binding are blocked with excess protein (i.e., BSA, Pierce Chemical ~~SuperBlock~~ SUPERBLOCK chemical preparation), approximately 10^{11} phage are added to each well. After several hours incubation at 4°C, the liquid is discarded from the wells with 200 mM glycine (pH2) which denatures the protein-phage complex. Bacteria are infected with the released phage after the pH is neutralized and cultured overnight. The infected cells release phage, ~1000 per minute per bacterium, so that the titer of the final culture is 10^{12} plaque forming units per ml. This constitutes one round of screening. The process is repeated three times in series, and the resulting phage are grown as isolates. We anticipate that the peptide ligand preferences for the individual DD's will vary, as DDs are < 31 % identical in amino acid sequence. Definition of an optimal DD peptide ligand will be useful in computer searches (http://expasy.hcuge.ch/sprot/scnpsit2.html) of possible cellular ligands. In addition, if we are successful in identifying a motif, we will generate an additional biased peptide libraries, as we did for SH3 (Sparks *et al.*, 1996a) and WW (unpublished) peptide ligands, which should accelerate defining the peptide ligand specificity of other DD's in the future.

Please replace the paragraph at page 131 beginning at line 23 with the following amended paragraph:

As mentioned above, the ER is organized into distinct domains (Figure 22). Several of these regions contain sites predicted to be useful for drug intervention of ER function. For instance, the estrogen receptor and other nuclear receptors interact with one or more steroid receptor co-activators (SRC's) via a conserved alpha-helical domain located with in the AF2 region of the receptor. This interaction is ligand dependent and is believed

to bridge the interaction between the receptor and transcriptional activation. Many co-activators have been identified and their interactions with several nuclear receptors have been investigated. Mutagenesis experiments have identified the sequence of the binding domain on the co-activator as an LXXLL (SEQ ID NO:121) motif where X is any amino acid. This sequence has been shown to be both necessary and sufficient for receptor binding (Heery *et.al*, 1997; Torchia *et.al*, 1997). The XX portion of this motif and the surrounding residues are believed to impart specificity to the interaction. Disruption of this interaction would be predicted to disrupt receptor signaling by blocking transcriptional activation. Peptides that mimic this interaction will provide a tool for discovering pharmacological agents that act at the co-activator binding site on the ER. Additional sites on the ER that could serve as targets for drug intervention include the AF1 region, the dimerization domain and the DNA binding domain. Once peptides are obtained for one or more of these sites, they can be used in a competitive displacement assay to screen libraries of compounds.

Please replace the paragraph at page 132 beginning at line 12 with the following amended paragraph:

The ER (Panvera Corp.) was immobilized on ~~Immulon 4~~ IMMULON 4 microtitration test plates (Dynatech) for the phage affinity selection, after it was determined that the immobilized ER was capable of binding estradiol. Phage display was conducted on the ER, as described in example 1 above, in both the presence and the absence of the natural ligand for the ER, 17-β estradiol (100 μM).

Please replace the Table at page 136 with the following amended Table:

Table 11-1

Cluster Analysis for Estrogen Receptor

	Phage #
S R T W E S P L G T W E W S R (SEQ ID NO:122)	13
S S K Y S Y S R S S E G H S R (SEQ ID NO:123)	29
S S **W V R L S D F P W G V** S R (SEQ ID NO:124)	1
S S **W** D **R L S D F P W G V** S R (SEQ ID NO:125)	2
S S **W I R L R D L P W G** E S R (SEQ ID NO:126)	3
S S **W V** L **L R D L P W G** S R (SEQ ID NO:127)	31
S S **C K W Y E** K **C** S G L W S R (SEQ ID NO:128)	7
S S G I **C F F W D** G **C** F E S R (SEQ ID NO:129)	35
S R N L **C F F W D** D E Y **C** S R (SEQ ID NO:130)	41
H H H R H P A H P H T Y G G (SEQ ID NO:131)	47

Please replace the Table at page 137 with the following amended Table:

Table 11-2

Cluster Analysis for ER + Estradiol

	Phage #
S R A G L L S D L L E G K S R (SEQ ID NO:132)	1/2
S S R S L L R D L L M V D S R (SEQ ID NO:133)	6
S S N K L L Y N L L K M E S R (SEQ ID NO:134)	22
S S K S L L L N L L S T P S R (SEQ ID NO:135)	23
H S F P P E S L L V R L L Q G G (SEQ ID NO:136)	42
S R L E M L L R S E T D F S R (SEQ ID NO:137)	3
S R L E E L L K W G S V T S R (SEQ ID NO:138)	11
S R L E Q L L K E E F S Y S R (SEQ ID NO:139)	21
S R L E Q L L R S E P D F S R (SEQ ID NO:140)	27
S R L E D L L R A P F T T S R (SEQ ID NO:141)	28
S R L E S L L R F G Q L D S R (SEQ ID NO:142)	29
S S R L L S L L V G D F N S R (SEQ ID NO:143)	19/20
S R L E E L L L G T N R D S R (SEQ ID NO:144)	30
S R L K E L L L L P T D L S R (SEQ ID NO:145)	15
S R L E C L L E G R L N C S R (SEQ ID NO:146)	34
S S K L Y C L L D E S Y C S R (SEQ ID NO:147)	35
S R L S C L L M G F E D C S R (SEQ ID NO:148)	36
S S K L I R L L T S D E E L S R (SEQ ID NO:149)	37
S S R L M E L L Q E G Q G W S R (SEQ ID NO:150)	40
S S N H Q S S R L I E L L S R (SEQ ID NO:151)	4
S S R L W Q L L A S T D T S R (SEQ ID NO:152)	16
S S N S M L W K L L A A P S R (SEQ ID NO:153)	13/14
S S K T L W R L L E G E R S R (SEQ ID NO:154)	17
S R A G P V L W G L L S E S R (SEQ ID NO:155)	32

Please replace the Table at page 138 with the following amended Table:

Table 11-3

Additional Sequences from ER + Estradiol

	Phage #
S S L T S R D F G S W Y A S R * (SEQ ID NO:156)	5
S S W V R L S D F P W G V S R * (SEQ ID NO:157) (also isolated (-) estradiol)	24/25
S S E Y C F Y W D S A H C S R * (SEQ ID NO:158)	33
S R S L L E C H L M G N C S R (SEQ ID NO:159)	7
S S E L L R W H L T R D T S R (SEQ ID NO:160)	8
S R L E Y W L K W E P G P S R (SEQ ID NO:161)	12
S R S D S I L W R M L S E S R (SEQ ID NO:162)	31

S S K G V **L** W R M **L** A E P V S R (SEQ ID NO:163)	38/39
H S H G P **L** T L N **L** L R S S G G (SEQ ID NO:164)	41
S S A G G G A P A G S T P S R (SEQ ID NO:165)	26
S S Y Q W E T H S D K W R S R (SEQ ID NO:166) (weak binder)	10
S S V T K K A L T I A K D S R (SEQ ID NO:167) (weak binder)	18

*binding not inhibited by antiestrogens

Please replace the paragraph at page 139 beginning at line 12 with the following amended paragraph:

Ex	Target	Peptide
1	HCMV UL44	E-H-V-C-S-W-G-W-G-R-C (SEQ ID NO:168) D R L T K (SEQ ID NO:169) N K I A H (SEQ ID NO:170) Q M G (SEQ ID NO:171)
2	Protein Kinase CβII	W-Phi-C-Pho-G-X-(F/L)-C and W-T-C-(V/I)-N- C
3	human MDM2	S-F-T-D-Y-W-R-D-L-E-Q (SEQ ID NO:172) and conservative mutants thereof.
5	tyrosine tRNA	Y-Phi-W-P-W and Y-Phi-W-P-Phi and (Y/F)-(S/T/G/A/H)-W-P(W/G/D/S/P) and (Y/F/W/L)-W-W-P-(D/E/S/N)-W-G
8	glucosidase	{F-K}-P-W-P-(I/V)-Y {}=optional
	carboxypeptidase	P-G-W-W (SEQ ID NO:173)
	ProRS	S-R-D-W-G-F-W (SEQ ID NO:174) E (SEQ ID NO:175)
11	Estrogen Receptor	W-Pho-R-L-Phi-D-Pho-P-W-G and C-F-F-W-D (SEQ ID NO:176) and L-X-X-L-L (SEQ ID NO:177)